UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF
CHEMICAL SAFETY
AND POLLUTION
PREVENTION

December 3, 2012

Memorandum

Subject: Protocol Review for Rug Doctor Carpet Sanitizer;
EPA Reg. No. 49158PA1, DP#405312

From: Lorilyn M. Montford LM 12/4/12 for Emily Mitchell
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Antimicrobials Division (7510P)

Thru: Emily Mitchell, Chief
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Applicant: Rug Doctor, Inc.
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I BACKGROUND

In an effort to add a sanitization claims to their carpet cleaner product, Rug Doctor (EPA Reg. No. 49158PA1), has submitted a test protocol for Agency review. The protocol was developed by ATS Labs, located at 1285 Corporate Center Drive, Suite 110, Eagan, MN 55121.

II USE DIRECTIONS

The product provides use directions for general cleaning and one-step sanitization for carpets that are made from natural and synthetic fibers. The use directions state that the product is not to be used on wool carpet. Directions on the proposed label state the following:

- Directions For General Cleaning: 1- Remove any excess solids, liquid or dirt. 2- Spray area generously. 3 – Lightly scrub the stained area working toward center from edges. 4- Blot with paper towel, pressing deep into the stain until dry.

- Directions For One-Step Cleaning and Sanitizing: 1- Remove any excess solids, liquid or dirt. 2- Hold the spray bottle approximately 3 inches from the surface of the carpeting and dispense around XX mLs of product per 12x12 inch area to be sanitized. 3- Apply moderate to heavy pressure and use a scrub brush to agitate the area. 4- Let product stand for 1 hour. 5- Blot with paper towel, pressing deep into the stain until dry.*

- * - The applicant has added a statement explaining that an additional step may be added to further rinse the product off the carpet...."Something along the lines of # of blot again with a clean damp white cloth to remove any residue or leftover product."

III AGENCY STANDARDS

The effectiveness of sanitizers for non-food contact surfaces must be supported by data that show that the product will substantially reduce the numbers of test bacteria on a treated surface. Testing requirements in EPA DIS/TSS-10 may be used. The test surface(s) should represent the type(s) of surfaces recommended for treatment on the label, i.e., porous or nonporous. Tests should be performed with each of 3 product samples, representing 3 different product lots, one of which is at least 60 days old against *Staphylococcus aureus* (ATCC 6538) and either *Klebsiella pneumoniae* (aberrant, ATCC 4352) or *Enterobacter aerogenes* (ATCC 13048 or 15038). Results must show a bacterial reduction of at least 99.9% over the parallel control within 5 minutes. These Agency standards are presented in DIS/TSS-10.

IV BRIEF DESCRIPTION OF PROTOCOL

Objective:
This protocol is designed to evaluate the antimicrobial efficacy of a carpet spray spot treatment application sanitizer on carpets (soft surfaces). For sanitizer products intended for use on soft, non-food contact surfaces, a carpet sanitizer methodology is used to generate efficacy data. The test system proposed is a modification of the AOAC methods prepared by the Registration Division, Office of Pesticide Programs, EPA, 1976 and revised in 1981.

Protocol Synopsis:
Test organism cells dried on carpet squares are exposed to the test substance for a specified exposure time. After exposure, the carpet squares are transferred to vessels containing neutralizer and assayed for survivors. Appropriate population controls, culture purity, sterility and neutralization confirmation controls are performed.

Carrier Preparation:
Two carpet types were recommended for use: nylon and polypropylene carpet. Alternate carpet types may be recommended. The sponsor is responsible for selecting the appropriate carpet to be used in the test. The carpet selected will be cut into approximately 8 inch x 12 inch pieces. Six approximately 2 inch x 2 inch square carriers will be cut into the carpet. The carpet is to be fastened to a mounting tray (or equivalent) and will be autoclave sterilized for $\geq$ 20 minutes prior to use in testing.

Inocula Preparation:
Staphylococcus aureus and *Enterobacter aerogenes* cultures will be obtained from the American Type Culture Collection (ATCC). The test organisms will be transferred daily on Nutrient Agar A slants for $\geq$3 but $\leq$ 30 transfers. The growth will be washed from a 24 $\pm$ 2 hour

Nutrient Agar A slant using 5.0 mL of phosphate buffer dilution water (PBDW). The 0.01% Triton will be omitted from the diluent to avoid the potential for interaction with the test substance. The growth will be aspirated and transferred to 99 mL PBWD. A 2.0 mL aliquot of this suspension will be added to sufficient Nutrient Agar B bottles. The inoculum will be evenly distributed within the bottles and the excess inoculum will be removed. The bottles will be incubated, agar-side down, for 18-24 hours. Following incubation, approximately 3 mL of PBDW will be added to each bottle and the inoculum will be collected. Approximately 15-20 sterile glass beads will be added to the bottles to aid in recovery. The growth suspension will be removed and filtered through sterile gauze or sterile Whatman #2 filter paper pre-wetted with 1.0 mL of PBDW. Similar to AOAC 960.04 to ensure that any agar harvested with the organism is removed from the test suspension, the growth suspension will be filtered through sterile gauze or Whatman #2 filter paper pre-wetted with 1.0 mL of PBDW. The test culture may be further adjusted, where appropriate to target approximately $1x10^8 - 1x10^{10}$ CFU/mL. This bacterial target range is recommended based on ATS' history of conducting this methodology. McFarland standards or a spectrophotometer may be used to aid with culture adjustment. An organic soil load can be added per Sponsor request.

Preparation of Test Substance:
The test substance is prepared according to the directions for intended use of the product. This product appears to be a ready to use product. If a dilution of the test substance is requested by the Sponsor, the diluted test substance shall be used within three hours of preparation.

Inoculation of Test and Control Carriers:
No information provided in the draft protocol describing how bacterial stock suspension (in mL) is to be inoculated onto carpet (piles) squares. In addition, information concerning incubation, time, and humidity should be noted in the protocol.

Treatment of Inoculated Test Carriers:
After drying, apply the test substance as specified by the Sponsor. Scrub each carpet carrier for approximately 30 seconds using approximately 30 circular clockwise strokes and approximately 30 circular counterclockwise strokes using a 4 1/4 x 1 5/8 in. surgical hand brush with ½ in. bristles. A circular area of pile approximately 3 inches in diameter around the center of each carrier will be scrubbed using this treatment. Moderate to heavy pressure will be applied downward on the brush to work the solution to the base of the pile. A new sterile brush will be utilized for each carpet square. A calibrated timer will be started upon application of the test substance and staggered intervals will be followed to treat subsequent carpet carriers. The treated and scrubbed carpet will remain at room temperature, uncovered, for the Sponsor specified exposure time. Exposure begins once the test substance has been applied. Following the Sponsor specified exposure time, remove each carrier from the larger carpet piece. Transfer each carpet carrier to individual vessels containing approximately 100 mL of neutralizer broth and 10 stainless steel penicylinders; this represents a 10^0 dilution. Care must be taken to place the carpet side down in the neutralizer vessel. Each vessel is to be shaken for at least one minute, at approximately 200 RPM, to free the bacteria from the carpet fibers. Ten-fold serial dilutions will be prepared. A 1.0 mL aliquot of the 100 – 10-3 dilutions will be plated in duplicate onto the appropriate agar.

Incubation and Observation:
Incubate the *Staphylococcus aureus* plates at 35-37°C for 48± 4 hours. Incubate the *Enterobacter aerogenes* plates for 48±4 hours at 25-30°C. If necessary, the subcultures may be placed at 2-8°C for up to three days prior to examination. Following incubation, the subcultures will be visually examined for growth. If possible, count plates containing between

30 and 300 CFU. Representative test plates will be stained and/or biochemically assayed to confirm or rule out the presence of the test organism.

V. CONCLUSIONS

1. The following issues must be resolved before the test protocol is considered acceptable:

(a) If this product is intended to be a "spot treatment" it needs to be specified on the label. In addition, the surface area intended for treatment should be included on the label.

(b) Under the "Use Directions" section, directions are given to "*spray generously*". This direction is very vague, and more specific information needs to be provided explaining the amount of product to be used. For example, no. of pumps of spray so many inches from the surface" or an amount in mL needs to be provided.

(c) More information needs to be provided on where the product may be used. If the product is intended to be used in a hospital or medical institution, a wet vacuum pickup must be specified in the label directions for use.

(d) On page 1 of the proposed label, Directions for "one-step" cleaning can only be supported if testing is conducted in the presence of an organic soil load.

(e) The statement, "odor-causing bacteria" on the proposed label is considered a non-public health claim. Therefore, no killing of bacteria should be mentioned with this statement. Also remove "99.9%" language from being used in conjunction with "odor-causing bacteria'.

(f) On page 2 of the proposed label, the applicant lists the types of carpet the product is to be used on: natural and synthetic fibers. The applicant must define what materials will be used to support what "natural" refers to as well as define the types of blends the product is intended for, i.e., natural or synthetic carpet blend the product is to be use on, i.e., 80/20 nylon/acrylic, 70/30 nylon/cotton/acrylic.

(g) Preparation of the test organism appears acceptable. The applicant needs to provide more information on the amount of product to be used in the "Exposure Conditions" portion of the protocol.

(h) In the, "Test System Recovery Section" of the protocol, the applicant needs to provide an "exposure time" for the product.

(I) Under "Directions for Use", directions should explain to blot after an amount of time has passed. Also, explaining "to press deep into stain" should possibly be followed by "until dry".

A clear formula for the product showing active ingredients must be provided.